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Full Research Paper

Polyphenolic Profile and Antioxidant Activities of Oolong Tea Infusion under Various Steeping Conditions

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Abstract The phenolic profile and antioxidant activities of oolong tea extract were investigated after tea was steeped in 90 or 100 °C water for 3 or 10 min. The extraction yield increased with increasing temperature and extended steeping time. However, higher temperature and longer time (100 °C water for 10 min) led to loss of phenolics. The aqueous extract of oolong tea (AEOT) at 100 °C for 3 min exhibited the strongest antioxidant activity. The major polyphenolic components of the AEOT were identified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin-3-gallate (ECG). The two major catechins (EGC and EGCG) in the tea infusion contributed significantly to the investigated antioxidant activities [i.e., the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging and superoxide radical scavenging activities] with high correlation values in $r = 0.9486$ and 0.9327 for the EGC and $r = 0.9592$ and 0.8718 for the EGCG, respectively.

Keywords: Oolong tea; Infusion; Antioxidant activity; Phenolics

1. Introduction

Oxidation is an essential biological process for energy production in many living organisms. However, excessive reactive oxygen species, produced *in vivo* during some oxidative reactions [1], are not only strongly associated with lipid peroxidation but also involved in the development of a variety of physiological conditions including cellular aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes and neurodegeneration [2,3]. Although the human body possesses an inherent antioxidant defense system to protect against oxidative damage, it is unable to entirely prevent the damage caused by reactive oxygen species. Recently, there have been increasing reports that some edible plants may afford protection and/or treatment of some chronic diseases. Generally, these beneficial effects are attributed to their antioxidant constituents, including vitamin C, vitamin E, carotenoids, flavonoids, catechins, anthocyanins, etc. [4,5].

Tea is one of the most commonly consumed beverages in the world for its desirable aroma, taste and putative positive physiological functions [6]. Tea is rich in flavonoids and other polyphenols that have been shown to possess a wide range of biological and pharmaceutical benefits, including anticarcinogenic, antioxidative, and hypolipidemic activities [7,8]. These beneficial effects are may be attributed to tea's antioxidant activity, e.g., free radical scavenging and metal chelating abilities. Depending on the manufacturing process, teas are classified into three major types: non-fermented green tea, semi-fermented oolong tea and fermented black teas [9]. Oolong tea is subjected to a moderate level of enzymatic oxidation during processing and drying [10]. Yen and Chen reported that oolong tea exhibited a stronger antimutagenic activity than green or black tea [11]. However, Ohe *et al.* suggested [12] that catechins were not the exclusive components of oolong tea responsible for the anti-genotoxic effect against nitroarenes.

In China and Southeast Asia, oolong tea is usually prepared by soaking the tea in hot water (> 90 °C) using a covered ceramic pot [13]. Carefully followed stirring and steeping procedures using appropriate time and temperature are critical to extract catechins or theaflavins from teas [14,15]. For example, high water temperature could lead to loss of unstable polyphenolic components after 10–15 min of steeping [16]. Our previous work demonstrated that infusion conditions had a major effect on antioxidant potentials and sensory assessment of oolong tea [17]. However, little information on the effect of steeping conditions on individual polyphenolic component and the relation to antioxidant activities is available. Based on the previous results, the polyphenolic profile and antioxidant activities of oolong tea infusion under different infusion conditions were investigated in order to improve the drink preparation in this study.

2. Results and Discussion

2.1 Extraction yield of oolong tea

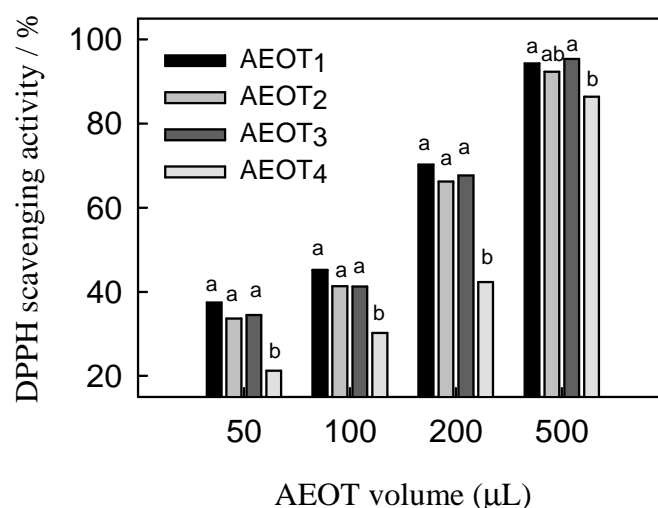
Aqueous extract of oolong tea (AEOT) was prepared by infusing 5.0 grams of tea in 200 mL of 90 or 100 °C distilled water in thermostatic water bath for 3 or 10 min. The extract was filtered and then evaporated in vacuum at below 70 °C in a rotary evaporator. The amount of tea powder extracted were

0.974, 1.086, 1.065 and 1.136 g for AEOT₁ (90 °C, 3 min), AEOT₂ (90 °C, 10 min), AEOT₃ (100 °C, 3 min) and AEOT₄ (100 °C, 10 min), respectively, exhibiting a tendency of increase with increasing infusion temperature and time. The similar result was found in the previous study [17].

2.2 DPPH radical scavenging activity of oolong tea infusion

It is known that antioxidants inhibit lipid peroxidation by their free radical scavenging activity [1,18]. The DPPH radical scavenging activity has been extensively used for screening plant extract antioxidants [19]. Figure 1 shows the DPPH radical scavenging activity of AEOT under different infusion conditions. The AEOT exhibited the DPPH radical scavenging activity in a dose-dependent manner. When an amount of 100 µL of the aqueous extract was used, the DPPH radical scavenging activities of AEOT₁, AEOT₂, AEOT₃ and AEOT₄ were 45.23, 41.35, 41.32 and 30.28 %, respectively. The strong DPPH scavenging activity of these tea infusions can be attributed in part to catechins present in oolong tea or possibly to some other polyphenols with low molecular weight [20]. However, increasing infusion temperature and time (100 °C, 10 min) resulted in a decrease of the DPPH radical scavenging activity, especially when 50–200 µL of the aqueous extract were used, which might be related to loss of polyphenolic components (Table 1).

Figure 1. Effects of AEOT under different steeping conditions at various concentrations of oolong tea aqueous infusion on the DPPH radical scavenging activity. Different letters indicate significant differences between treatments according to Tukey multiple comparisons ($p < 0.05$).



2.3 Superoxide radical scavenging activity of oolong tea infusion

Superoxide radicals are produced by a number of cellular reactions associated with various enzyme systems, such as lipoxygenases, peroxidase, NADPH oxidase and xanthine oxidase. Superoxide anions play important roles in plant tissues and are involved in the formation of other cell-damaging free radicals [1]. As shown in Figure 2, a strong superoxide anion scavenging activity (>80% inhibition)

was observed when 0.1 mL of the aqueous extract of oolong tea was used. Furthermore, lower superoxide radical scavenging activity was observed in AEOT₄ prepared under the infusion condition of 100 °C water for 10 min, compared with AEOT₁ and AEOT₃, when 50 µL and 100µL of their extracts were used, respectively.

Figure 2. Effects of AEOT under different steeping conditions at various concentrations of oolong tea aqueous infusion on the superoxide radical scavenging activity. Different letters indicate significant differences between treatments according to Tukey multiple comparisons ($p < 0.05$).

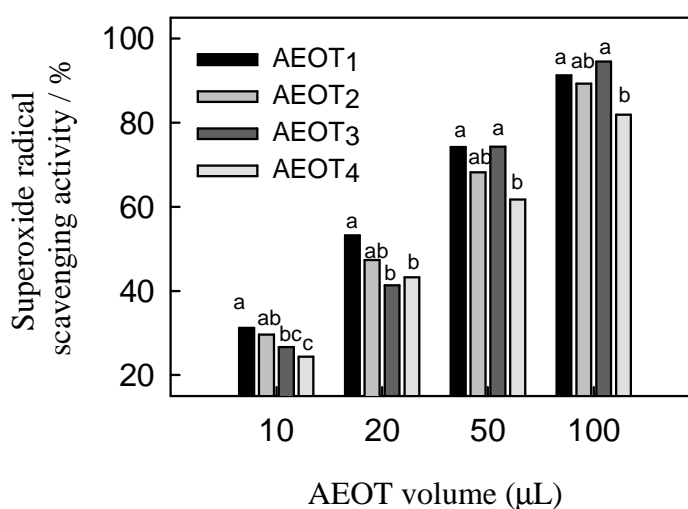


Table 1. Total phenolic content and free radical scavenging activities of AEOT.

| Sample | Polyphenolic content (mg of GAE/mL) ^a | DPPH radical scavenging activity (EC ₅₀ µL) ^b | Superoxide radical scavenging activity (EC ₅₀ µL) ^c |
|-------------------|---|---|---|
| AEOT ₁ | 2.83 ab | 119.1 b | 24.4 c |
| AEOT ₂ | 2.78 abc | 134.8 b | 31.1 ab |
| AEOT ₃ | 2.93 a | 115.4 b | 21.2 c |
| AEOT ₄ | 2.65 c | 251.0 a | 33.5 a |
| Vc ^d | | 91.0 | 17.4 |

Different letters in the same column indicate significant differences between treatments according to Tukey multiple comparisons ($p < 0.05$). ^a. Expressed as mg of gallic acid equivalents (GAE) per mL of AEOT; ^b. Half effective dose (EC₅₀) to scavenge DPPH radicals in 2.9 mL of 0.1 mM DPPH solution; ^c. Half effective dose (EC₅₀) to scavenge superoxide radicals in the reaction system (1.3 µmol/L riboflavin, 13 mmol/L methionine, 63 µmol/L nitro blue tetrazolium and 100 µmol/L EDTA, pH = 7.8, 3 mL) and ^d. Vitamin C (Vc) at 2 mg/mL was used as a positive control.

2.4 Effects of various infusion conditions on total phenolic content

As shown in Table 1, a lower total phenolic content was observed in AEOT₄, compared with AEOT₁ and AEOT₃. It is suggested that steeping oolong tea at higher temperature and/or longer time

(100 °C water for 10 min) may lead to loss of phenolics and reduced antioxidant activities. Standley *et al.* [21] found that the reduced antioxidant potency of tea samples at various processing stages was due to a decrease in the total polyphenolic content.

2.5 Major catechins in oolong tea infusion

Major bioactive components of teas usually include polyphenols, particularly the catechins [7, 8]. In this study, (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC) and (-)-epicatechin-3-gallate (ECG) were detected in all AEOT samples. The highest concentrations of EGCG, EGC, C, EC, and ECG were 18.122 mg/100mL in AEOT₁, 29.892 mg/100mL in AEOT₃, 0.9 mg/100mL in AEOT₃, 4.4 mg/100mL in AEOT₃ and 14.853 mg/100mL in AEOT₄, respectively (Table 2), which also confirmed the previous report that EGCG, EGC and ECG were the major catechins in fermented teas [22]. It was noted that the contents of EGCG and EGC significantly decreased ($p<0.05$) under the conditions of higher temperature and longer steeping time (100 °C, 10 min), which might be related to degradation or transformation of catechins. Manach *et al.* reported that monomeric catechins could be easily transformed to theaflavins and thearubigins through oxidation and polymerization during fermentation and heating of black tea [23].

Table 2. Catechin contents in AEOT under various steeping conditions (expressed as mg/100mL).

| Sample | EGCG | EGC | C | EC | ECG |
|-------------------|----------|---------|-------|--------|---------|
| AEOT ₁ | 18.12 a | 28.44 a | 0.6 a | 2.6 b | 7.32 b |
| AEOT ₂ | 15.79 ab | 26.11 a | 0.8 a | 3.4 ab | 10.36 b |
| AEOT ₃ | 17.52 a | 29.89 a | 0.9 a | 4.4 a | 14.33 a |
| AEOT ₄ | 12.46 b | 21.44 b | 0.7 a | 3.1 ab | 14.85 a |

Different letters in the same column indicate significant differences between treatments according to Tukey multiple comparisons ($p<0.05$)

Table 3. Correlation coefficients between antioxidant activity and individual catechin of AEOT.

| | Correlation coefficients (r) ^a | |
|------|--|--|
| | DPPH radical scavenging activity (EC ₅₀) | Superoxide radical scavenging activity (EC ₅₀) |
| EGCG | 0.9592 | 0.8718 |
| EGC | 0.9486 | 0.9327 |
| C | 0.2646 | 0.2828 |
| EC | 0.2646 | 0.4359 |
| ECG | 0.5558 | 0.1732 |

^a In the linear regression analysis, polyphenolic content was regarded as X and EC₅₀ of radical scavenging activity as Y.

2.6 Relationship of polyphenolic profile and antioxidant activity of AEOT

Oolong tea infusion exhibited a strong free radical scavenging activity (Table 2). For instance, the EC₅₀ value of AEOT₃ to scavenge the DPPH and superoxide radicals were 115.4 and 21.2 µL of tea infusions, respectively, which were 126 % and 122 % of the EC₅₀ of 2 mg/mL Vitamin C (Vc) used as a positive control (Table 1). Moreover, there are strong correlations between the antioxidant activities and some catechins. EGCG exhibited high correlation values in $r = 0.9592$ and 0.8718 with its DPPH radical scavenging ability and superoxide radical scavenging ability, respectively, while EGC also presented high values in $r = 0.9486$ and 0.9327 , respectively (Table 3). However, other three catechins, e.g., C, EC and ECG only showed much lower correlations with their antioxidant capabilities (Table 3). Thus, EGCG and EGC are considered the more important and major contributors to the antioxidant activities of the oolong tea.

3. Conclusions

The aqueous extract of oolong tea exhibited strong radical scavenging activities. The scavenging ability of AEOT against the DPPH and superoxide radicals depended on the steeping temperature and time. Oolong tea soaked in 100 °C water for 3 min showed the highest activities of scavenging DPPH and superoxide radicals. The strong radical scavenging activity of AEOT was associated with a high content of catechins which include EGC, EGCG, ECG, EC and C. In addition, it was found that EGCG and EGC in the oolong tea infusion significantly contributed to the investigated antioxidant activities.

4. Experimental Section

4.1 Plant materials

Oolong tea (*C. sinensis*) (commercial name: Fenghuangdancong) was obtained from Funan Oolong Tea Corporation of Chaozhou, Guangdong province in April, 2004. Preparation for oolong tea involved 1–2 h of solar withering, 4 h of indoor withering, 5 cycles of 20-min hand rolling, followed by 2 h of fermentation, 5 min of steaming, and 5 h of drying at 90 °C. The oolong tea was packaged in a gas-tight plastic bag and stored in a refrigerator (4 °C) until extraction.

4.2 Reagents

HPLC grades of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin-3-gallate, and caffeine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, glacial acetic acid, and hydrochloric acid (37 % v/v) used for the mobile phase and the tea extraction were obtained from Merck Co. (HPLC grade reagent). All other reagents used were analytical grade.

4.3 Sample preparation

AEOT was prepared based on the traditional practice used in Chaozhou region. Oolong tea (5 g) was quickly washed with distilled water (200 mL) at 80 °C for 10 s in a covered ceramic pot. After removal of the washing water, the tea leaves were soaked with 90 or 100 °C distilled water (200 mL) in a thermostatic water bath for 3 and 10 min (Table 1). The tea infusion was filtered through Whatman No. 1 filter paper, and then collected. The AEOT was diluted to 1000 mL with distilled water, and then separated into five equal solutions (200 mL). Two solutions were dried below 70 °C under reduced pressure on an Eyela CA-1111 Rotavapor (Tokyo, Japan) to give a crude powder, which was then re-dissolved in methanol for HPLC analysis. The other three parts were prepared for determination of total phenolic content and antioxidant activities.

4.4 Scavenging DPPH[•] radical activity

The scavenging activity of AEOT against the DPPH[•] radicals was assessed according to the method of Larrauri *et al.* [24] with some modifications. Briefly, different amounts of AEOT solutions (50 to 500 µL) were mixed with DPPH in methanol solution (0.1 mM, 2.9 mL). Distilled water was then added to maintain the final volume of 3.5 mL. After the solution was incubated for 30 min at 25 °C in dark, the absorbance was measured at 517 nm. The control sample contained methanol instead of the AEOT while the blank contained methanol instead of DPPH solution. In the experiment, *L*-ascorbic acid (Vc) was used as a positive control. The inhibition of DPPH[•] radicals by AEOT sample was calculated by the following equation: DPPH[•] radical scavenging activity/% = $[1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100$. Three replicates were carried out to determine the scavenging activity of AEOT against the DPPH[•] radicals.

4.5 Superoxide radical scavenging activity

Determination of the superoxide radical scavenging activity was conducted according to the method described by Siddhurajua *et al.* [25]. All solutions were prepared in 0.05 mol/L phosphate buffer (pH = 7.8). The photo-induced reactions were performed in an aluminium foil-lined box with two 30 W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until luminous intensity reached about 4,000 lux. Various amounts of AEOT solutions (10 to 100 µL) were mixed with a reaction buffer solution (1.3 µmol/L riboflavin, 13 mmol/L methionine, 63 µmol/L nitro blue tetrazolium and 100 µmol/L EDTA, pH = 7.8) to the final volume of 3 mL. The reaction solution was illuminated for 15 min at 25 °C. The reaction mixture without AEOT sample was used as a control. The superoxide radical scavenging activity was calculated by the following formula: $(1 - A_{\text{sample}} / A_{\text{control}}) \times 100$. Three replicates were performed to analyze scavenging activity of AEOT against the superoxide radical.

4.6 Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent by the method of Singleton and Rossi [26], using gallic acid as a standard. Results were expressed as mg of gallic acid equivalents (GAE) per gram on a dry weight basis.

4.7 High performance liquid chromatography

Analysis of AEOT was carried out by a Waters Alliance 2695 high-performance liquid chromatograph (Milford, MA, USA) equipped with a XTerra RP-C₁₈ column (3.9 × 100 mm, 3.5 µm particle size, Waters). An acidified acetonitrile–water mobile phase with a flow rate of 0.7 mL/min at 25 °C, was used for a gradient elution: 0–30 min: 0–15 % B (acetonitrile/water, 1/1 v/v, pH 2.6) in A (acetonitrile/water, 1/9 v/v, pH 2.6); 30–50 min: 15 % B in A; 50–60 min: 15–25 % B in A; 60–90 min: 25–100% B in A; and 90–100 min: 100–0% B in A. Calibration was made with authentic catechin standards (50 µg/mL) by plotting peak areas from the DAD absorbance signal at 280 nm versus catechin concentration [9]. In this analysis, the limits of quantification (LOQ) were 50.0, 300.0, 110.0, 65.5 and 14.0 ng/mL, while limits of detection (LOD) were 15.0, 90.0, 2.0, 20.0 and 4.5 ng/mL for EGCG, EGC, C, EC and ECG, respectively. Three replicates were performed.

4.8 Data analysis

All analyses were performed in triplicate. The data were analyzed by SPSS (Version 10.0). One-way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test any significant difference between the means. Differences between means at the 5 % level were considered to be significant.

Acknowledgements

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